

DATA EVALUATION RECORD
WHOLE SEDIMENT ACUTE TOXICITY, FRESHWATER INVERTEBRATES
OPPTS Guideline 850.1735

1. **CHEMICAL:** Permethrin PC Code No.: 109701

2. **TEST MATERIAL:** Permethrin technical Purity: 95.1%

3. **CITATION:**

Authors: Picard, C.R.
Title: 10-Day Toxicity Test Exposing Midges (*Chironomus dilutus*)
to Permethrin Applied to Formulated Sediment Under Static-
Renewal Conditions Following OPPTS Draft Guideline
850.1735.

Study Completion Date: June 29, 2010

Laboratory: Springborn Smithers Laboratories
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Sponsor: Pyrethroid Working Group
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Laboratory Report ID: 13656.6148

MRID No.: 48593615

DP Barcode: 396414

4. **REVIEWED BY:** Christie E. Padova, Staff Scientist, CSS-Dynamac Corporation

Signature: 

Date: 11/25/13

APPROVED BY: Teri S. Myers, Senior Scientist, CDM Smith

Signature: 

Date: 05/12/15

5. **APPROVED BY:**

Signature:

Date:

6. **STUDY PARAMETERS:**

Age of Test Organism: 3rd Instar, 11 days old
Definitive Test Duration: 10 days

Study Method: Flow-through
Type of Concentrations: Mean-measured sediment, bulk and OC-normalized

7. CONCLUSIONS:

Results Synopsis:

Based upon mean-measured sediment concentrations:

Survival:

LC₅₀: 275 µg ai/kg 95% C.I.: 229 to 329 µg ai/kg
Probit Slope: 2.57 (2.06 to 3.07)
NOAEC: 120 µg ai/kg
LOAEC: 220 µg ai/kg

Growth (AFDW):

EC₅₀: 92 µg ai/kg 95% C.I.: 69 to 124 µg ai/kg
Probit Slope: N/A
NOAEC: <54 µg ai/kg
LOAEC: 54 µg ai/kg

Based upon OC-normalized mean-measured sediment concentrations:

Survival:

LC₅₀: 13095 µg ai/kg TOC 95% C.I.: 10905 to 15667 µg ai/kg TOC
Probit Slope: 2.57 (2.06 to 3.07)
NOAEC: 5714 µg ai/kg TOC
LOAEC: 10476 µg ai/kg TOC

Growth (AFDW):

EC₅₀: 4381 µg ai/kg TOC 95% C.I.: 3286 to 5905 µg ai/kg TOC
Probit Slope: N/A
NOAEC: <2571 µg ai/kg TOC
LOAEC: 2571 µg ai/kg TOC

8. ADEQUACY OF THE STUDY:

A. Classification: Acceptable/Supplemental/Unacceptable

B. Rationale:

C. Repairability:

9. MAJOR GUIDELINE DEVIATIONS:

There were no deviations from OPPTS 850.1735 guidance that would affect the scientific soundness or acceptability of this study.

10. MATERIALS AND METHODS:**A. Test Organisms**

Guideline Criteria	Reported Information
Species: <i>H. azteca</i> or <i>Chironomus tentans</i>	Dipteran midge, <i>Chironomus dilutus</i>
Life Stage: For <i>C. tentans</i> : third instar (9-11 days old). The instar stage of midges must be confirmed by head capsule width (approx. 0.38 mm). For <i>H. azteca</i> : 7- to 14-day old amphipods must be produced. If growth is also an endpoint, a narrower range, such as 1- to 2-day old amphipods should be used.	3 rd instar, 11 days old At study initiation, the head capsule width of a sub-population of 20 larvae ranged from 0.35 to 0.45 mm (mean of 0.38 mm), and the dry weight of a sub-population of 20 larvae averaged 0.087 mg/larvae.
Supplier Brood stock can be obtained from laboratory, commercial, or government sources. (Sources obtained from the wild should be avoided unless cultured through several generations in the laboratory.)	Larvae were obtained from Environmental Consulting & Testing, Superior, WI.
All organisms from the same source?	Yes

B. Source/Acclimation

Guideline Criteria	Reported Information
Acclimation Period: The required culture and testing temperature is 23°C. The test organisms should be cultured in the same water to be used for testing.	The midges were acclimated for <i>ca.</i> 72 hours in water from the same source as water used during the definitive study. During acclimation, the water temperature ranged

Guideline Criteria	Reported Information
	from 23 to 24°C and the dissolved oxygen ranged from 6.8 to 9.2 mg/L.
Feeding:	Finely-ground flaked fish food suspension daily.
Pretest Mortality: A group of organisms should not be used if they appear unhealthy, discolored (eg <20% mortality 48 h before the beginning of a test).	No mortality, disease, or unusual behavior was observed in the 24- or 48-hour period immediately preceding the test.

C. Test System

Guideline Criteria	Reported Information
<p>Source of dilution water (overlying water) and sediment: Soft reconstituted water or water from a natural source. Tap water is acceptable if it is dechlorinated, deionized, and carbon filtered, but its use is not encouraged.</p> <p>Uncontaminated natural sediment is recommended.</p>	<p>Laboratory well water characterized as having a total hardness and total alkalinity as CaCO₃ of 68 and 18 to 20 mg/L, respectively, a pH range of 6.9 to 7.0, and a specific conductivity range of 430 to 450 µmhos/cm.</p> <p>Formulated sediment (Springborn Smithers Batch No. 012910) was prepared according to OECD Guideline 218 by mixing the following components (dw basis): 6.0% sphagnum peat, 20% kaolin clay, and 74% fine sand. While blending using a large-scale mixer, 6 L of laboratory well water was also added.</p> <p>Prior to use, the sphagnum peat was pre-soaked in dilution water for 7 days. During this time, the peat was amended with 120 g of powdered CaCO₃ to increase the pH from 3.3 to 6.0.</p>

Guideline Criteria	Reported Information
Does water support test animals without observable signs of stress?	Yes
Quality Of Water If problems are observed in culturing or testing of organisms, it is desirable to test water quality. Particulate, TOC, COD should be <5 mg/L and residual chlorine <11 µg/L	There were no apparent problems with water quality. On Day 0, the measured ammonia level (as N) in control sediment pore water was 15 mg/L.
Water Temperature 23°C for both species. The mean and instantaneous temperatures should not vary from the desired temperature by more than 1°C and 3°C, respectively.	Daily: 22 to 24°C Continuous: 22 to 25°C
pH Should not vary more than 50%. Survival is best at pH >6.5 for <i>C. tentans</i> .	6.9 to 7.4
Dissolved Oxygen Maintained between 40 and 100%.	4.4 to 7.9 mg/L (>40% saturation)
Total Hardness Should not vary more than 50%. <i>H. azteca</i> are sensitive to hardness (e.g., they are not found in waters with calcium at <7 mg/L and DO at <2 mg/L).	72 to 80 mg/L as CaCO ₃
Conductivity Should not vary more than 50%.	410 to 490 µmhos/cm

Guideline Criteria	Reported Information
<p>Sediment Characterization All sediment must be characterized for: pH, ammonia concentration of pore water, organic carbon content (total organic carbon (TOC)), particle size distribution, and percent water content.</p>	<p>Particle distribution – 79% sand, 4% silt, 17% clay (sandy loam; reviewer-derived from USDA soil texture triangle) TOC – 2.1% Percent solids – 65.45% pH – 6.8 CEC – not reported Bulk density – not reported</p>
<p>Additional Sediment Analysis BOD, COD, cation exchange capacity, Eh, pE, total inorganic carbon, total volatile solids, acid volatile sulfides, total ammonia, metals, synthetic organic compounds, oil and grease, petroleum hydrocarbons, and interstitial water analysis.</p>	<p>Not reported</p>
<p>Laboratory Spiked Sediment Material should be reagent grade unless prior evaluations dictate formulated materials, etc.; Must know the test material's identity, quantity of major ingredients and impurities, water solubility, estimated toxicity, precision and bias of analytical method, handling and disposal procedures.</p>	<p><u>Permethrin technical</u> Synonyms: FMC 33297, Pounce® IUPAC name: 3-phenoxybenzyl (1<i>RS</i>,3<i>RS</i>;1<i>RS</i>,3<i>SR</i>)-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate CAS name: (3-phenoxyphenyl)methyl 3-(2,2-dichloroethenyl)-2,2-dimethylcyclopropanecarboxylate Description: not reported Lot no.: PL07-0347 CAS No.: 52645-53-1 Purity: 95.1% Storage: room temperature in the dark Aqueous solubility: not reported</p>

Guideline Criteria	Reported Information
<p>Stock Solutions</p> <p>Test material should be dissolved in a solvent prior to mixing into test sediment; If solvent is used, both solvent control and negative control are required.</p>	<p>A 350-µg ai/mL primary stock solution was prepared by dissolving 0.03681 g test substance (0.03501 g ai) into 100 mL of acetone.</p> <p>From this, six individual dosing solutions were prepared by diluting the appropriate amount of stock solution into 25 mL acetone.</p> <p>All dosing solutions were clear and colorless, with no visible un-dissolved test substance.</p> <p>Negative and solvent controls were included in the test.</p>

Guideline Criteria	Reported Information
<p>Test Concentrations For Spiked Sediment For LC50 calculation, test concentrations should bracket the predicted LC50; sediment concentrations may be normalized to factors other than dry weight (e.g. organic content, acid volatile sulfides); Sediment may be mixed using rolling mill, feed mixer or hand mixer.</p>	<p>A jar-rolling technique was used to apply the test substance to the sediment. A 10-mL volume of the appropriate prepared dosing stock solution (in acetone) was applied to 0.0500 kg of fine silica and the solvent was allowed to evaporate off for 45 minutes. The dry sand was then added to 2.5 kg of wet sediment (total of 1.6863 kg dw) in individual glass jars. Each jar was then rolled for 4 hours at <i>ca.</i> 15 rpm. The jars were stored upright at 2 to 8°C for a 14-day equilibration period.</p> <p>Twice a week during the equilibration period and prior to being added into the replicate exposure vessels, the jars were mixed on the rolling mill for 2 hours to ensure the sediment was homogeneous.</p> <p>The range of nominal concentrations (63 to 2000 µg ai/kg) was based upon the results of a preliminary range-finding study.</p>
<p>Test Aquaria 1. <u>Material</u>: Glass or stainless steel or perfluorocarbon plastics. 2. <u>Size</u>: 300 ml high-form lipless beakers containing 100 ml of sediment and 175 ml of overlying water.</p>	<p>1. Glass and 40-mesh Nitex screen (for drainage) 2. 300 mL vessels containing 100 mL (<i>ca.</i> 4.0-cm layer) of sediment (equivalent to 102 g dw) and 175 mL of overlying water. The total overlying water plus sediment volume was maintained at <i>ca.</i> 275 mL.</p>
<p>Type of Dilution System Daily renewal or a flow-through system may be used.</p>	<p>Flow-through</p>
<p>Flow Rate 2 volume changes/day</p>	<p>2 volume additions/day</p>

Guideline Criteria	Reported Information
Aeration Dilution water should be vigorously aerated prior to use so that dissolved oxygen in the overlying water remains above 40% saturation.	None reported
Photoperiod 16 hours light, 8 hours dark at 500 to 1000 lux.	16 hours light, 8 hours dark; 510 to 960 lux
Solvents Use of a solvent should be avoided since they may influence the concentration in pore water. If used, it should not exceed 0.5 mL/L for static tests or 0.1 mL/L for flow-through tests. Acceptable solvents include triethylene glycol, methanol, ethanol, or acetone. Surfactants should not be used.	Acetone, 10 mL per 1.6863 kg dw sediment The acetone was allowed to completely evaporate during the mixing procedure.

D. Test Design

Guideline Criteria	Reported Information
Sediment Into Test Chambers One day prior (Day -1) to start of test: test sediment, reference sediment, and negative control sediment should be thoroughly homogenized and added to test chambers; Overlying water is added to chambers in a manner that minimizes suspension of sediment.	One day prior to the addition of chironomid larvae (day -1), the test systems were established. Overlying water was gently added, and each vessel was placed under the renewal system.

Guideline Criteria	Reported Information
<p>Renewal of Overlying Water: Renewal of the overlying water should be conducted on day -1 prior to the addition of organisms or food on day 0. For flow-through systems, the flow rates should not vary by more than 10% between any two chambers at any time. Proper operation should be verified by calibration prior to test initiation.</p>	<p>The overlying water was renewed via an intermittent delivery system in combination with a calibrated water-distribution system. The test system was calibrated before and after the test, and visually inspected at least twice daily for proper functioning.</p>
<p>Placing Organisms in Test Chambers: Should be handled as little as possible and introduced into overlying water below the air-water interface.</p>	<p>Midges were impartially assigned one or two at a time into intermediate test beakers until all beakers contained ten midges. The test was initiated when each intermediate beaker of midges was added to each respective test vessel.</p>
<p>Range Finding Test A definitive test will not be required if no toxicity is observed at concentrations of 100 mg/kg dry weight of sediment.</p>	<p><u>Preliminary toxicity assessment</u></p> <ul style="list-style-type: none"> • 10-day exposure at nominal levels of 0 (negative and solvent controls), 1.0, 10, 100, 1000, and 10000µg ai/kg • 10-day old larvae; three replicates per level, each containing 10 larvae • Survival averaged 93 (control), 87 (solvent control), 87, 97, 97, 43, and 0%, respectively • Ash-free dry weight (AFDW) averaged 1.65 (control), 1.06 (solvent control), 1.23, 1.49, 0.99, and 0.02 mg per larva, respectively
<p>Monitoring the test All test chambers should be checked daily and observations made to assess organism behavior such as sediment avoidance.</p>	<p>Test vessels were observed daily for mortality and abnormal behavior.</p>

Guideline Criteria	Reported Information
Nominal Concentrations of Definitive Test Control(s) and at least 5 test concentrations; dilution factor not greater than 50%. Concentrations above aqueous solubility may be used.	0 (negative and solvent controls), 63, 130, 250, 500, 1000, and 2000 µg ai/kg dw
Number of Test Organisms 10 organisms per test chamber are recommended. 8 replicates per treatment should be used.	80 larvae per level, with 10 larvae per replicate vessel and 8 biological replicates per level An additional 6 replicates per level were maintained for chemical analysis and pore water quality and analysis. The surrogate vessels did not contain organisms.
Test organisms randomly or impartially assigned to test vessels?	Yes
Feeding <i>C. tentans</i> in each test chamber are fed 1.5 ml of a 4 g/L Tetrafin ⁷ suspension daily. <i>H. azteca</i> may be fed with a mixture of yeast, Cerophy., and trout chow (YCT) at a rate of 1.5 mL daily per test chamber. A drop in DO. levels below 2.5 mg/L may indicate over-feeding and feeding should be suspended in all treatments until DO levels increase.	Midges were fed a finely-ground flaked fish food suspension (4.0 mg/mL) once daily at a rate of 1.5 mL/vessel.
Water Parameter Measurements Conductivity, hardness, pH, alkalinity, and ammonia should be measured in all treatments at the beginning and end of the test. DO should be measured daily. Temperature should be measured daily in one test chamber from each treatment. The mean and instantaneous temperatures should not vary from the desired temperature by more than 1 and 3°C, respectively.	Total hardness, alkalinity, specific conductance, and ammonia were measured in each treatment level and control solution from a composite sample at Days 0 and 10. Dissolved oxygen (DO), temperature, and pH were measured in each replicate vessel on Days 0 and 10, and in one alternating replicate from each level on Days 1 to 9. In addition, the temperature was continuously monitored in an auxiliary vessel in the

Guideline Criteria	Reported Information
	temperature-controlled water bath.
Chemical Analysis Needed if solutions were aerated, if chemical was volatile, insoluble, or known to absorb, if precipitate formed, if containers were not steel or glass, or if flow-through system was used. Concentrations should be measured in bulk sediment, interstitial water, overlying water, and stock solution.	Sediment from all levels was analyzed for permethrin on Days 0 and 10. Following removal of the overlying water, the sediment was centrifuged at <i>ca.</i> 1200 <i>g</i> for 15 to 30 minutes and extracted and analyzed using GC/MS based on methodology validated at Springborn Smithers (see Reviewer's Comments section for further details).

11. REPORTED RESULTS:

A. General Results

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	Yes (see Reviewer's Comments).
Control Criteria Was control mortality $\leq 30\%$? Were control <i>C. tentans</i> an average size of ≥ 0.6 mg.	Negative control: 6% Solvent control: 7% Negative control: 0.77 mg/larva Solvent control: 0.79 mg/larva
Percent Recovery of Chemical:	Results of quality control (QC) samples analyzed concurrently with test samples: <u>Sediment:</u> 79.7 to 102% of nominal

Guideline Criteria	Reported Information
Data Endpoints <ul style="list-style-type: none">- Survival- Dry weight (determined by pooling all living organisms from a replicate and drying at 60 to 90°C to a constant weight)- Body length (amphipod only)	<ul style="list-style-type: none">- Survival- AFDW
Raw data included?	Yes, sufficient

Effects Data

Toxicant Concentration				Survival		Ash-Free Dry Weight (AFDW) per Larva	
Nominal (µg ai/kg)	Mean-Measured ^(a)						
	Sediment (µg ai/kg)	Pore Water (µg ai/L)	Overlying Water (µg ai/L)	% ± SD	% Inhibition	mg ± SD	% Inhibition
Negative Control	<LOQ ^(b)	Not reported	Not assessed	94 ± 9	N/A	0.77 ± 0.11	N/A
Solvent Control	<LOQ	Not reported	Not assessed	93 ± 10	N/A	0.79 ± 0.09	N/A
Pooled Control	---	---	---	93 ± 9	N/A	0.78 ± 0.09	N/A
63	54	Not reported	Not assessed	91 ± 10	2	0.61 ± 0.14*	22
130	120	Not reported	Not assessed	95 ± 5	-2	0.32 ± 0.10*	59
250	220	Not reported	Not assessed	50 ± 14*	46	0.14 ± 0.08 ^(c)	82
500	490	Not reported	Not assessed	34 ± 22*	63	0.12 ± 0.09 ^(c)	85
1000	880	Not reported	Not assessed	8 ± 12*	91	0.09 ± 0.03 ^(c)	88
2000	2000	Not reported	Not assessed	0*	100	N/A	N/A

^(a) Results of the pore water analysis were not included in the study report and will be presented in a supplemental report, as stated by the study author. Overlying water was not analyzed in this study due to pyrethroids' strong affinity to sediment and regular renewal of the overlying water.

^(b) LOQ = 12 $\mu\text{g ai/kg}$.

^(c) Excluded from statistical analysis due to significant effect on survival at this level.

* Statistically-significant compared to the pooled control ($p < 0.05$).

Other Significant Results:

Biological: After 10 days, survival averaged 94 and 93% for the negative and solvent control groups, respectively, compared to 91, 95, 50, 34, 8, and 0% for the mean-measured 54, 120, 220, 490, 880, and 2000 µg ai/kg levels, respectively. Differences were statistically significant ($p < 0.05$) compared to the pooled control (93%) at the ≥ 220 µg ai/kg levels. Using mean-measured concentrations, the NOAEC and LOAEC for survival were 120 and 220 µg ai/kg, respectively, and the 10-day LC₅₀ (with 95% C.I.) was 430 (370 to 500) µg ai/kg.

Ash-free dry weights (AFDW) averaged 0.77 and 0.79 mg per larva for the negative and solvent control levels, respectively, and 0.61, 0.32, 0.14, 0.12, and 0.09 mg per larva for the mean-measured 54, 120, 220, 490, and 880 µg ai/kg levels, respectively. Differences were statistically-significant ($p < 0.05$) compared to the pooled control (0.78 mg/larva) at the 54 and 120 µg ai/kg levels. Higher treatment levels were not statistically assessed due to significant reductions in survival at these levels. Using mean-measured concentrations, the NOAEC and LOAEC for growth were < 54 and 54 µg ai/kg, respectively, and the 10-day EC₅₀ (with 95% C.I.) was 110 (90 to 130) µg ai/kg.

Analytical: Only results from sediment analyses were reported in this study. Permethrin concentrations changed slightly (-31 to +9.5%, reviewer-calculated) during the 10-day study. Overall, mean-measured concentrations represented 85 to 100% of nominal sediment concentrations.

B. Statistical Results

Statistical analyses were performed on midge survival and growth (ash-free dry weight, AFDW). Analyses were performed using the response values for each replicate test vessel within a treatment level. Percent survival data were arcsine square-root transformed prior to analysis.

A t-Test was used to compare the performance of the negative control and solvent control data. For both endpoints, data were statistically similar, and the treatment groups were compared to the pooled control data to determine potential treatment-related effects.

Normality of the data was evaluated using the Anderson-Darling Test (survival) or the Chi-Square Test (AFDW), and homogeneity of variance was evaluated using Bartlett's or Cochran's Tests at the 99% level of certainty. Both endpoints met these assumptions and were thus analyzed using Bonferroni's t-Test at a 95% level of certainty. NOAEC and LOAEC values were assigned based upon significance.

The Spontaneous Log-Log Analysis was used to calculate the LC₅₀ (with associated 95% confidence intervals), while the linear interpolation method was used to calculate the EC₅₀ (and associated 95% C.I.) for AFDW.

Analyses were performed using TOXSTAT Version 3.5 statistical software and mean-measured sediment concentrations.

Survival:

LC₅₀: 430 µg ai/kg

95% C.I.: 370 to 500 µg ai/kg

NOAEC: 120 µg ai/kg

LOAEC: 220 µg ai/kg

Growth (AFDW):

EC₅₀: 110 µg ai/kg

95% C.I.: 90 to 130 µg ai/kg

NOAEC: <54 µg ai/kg

LOAEC: 54 µg ai/kg

12. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method: The reviewer compared the negative and solvent control data using a two-sided t-test; no significant difference was detected for survival or dry weight. Normality was tested using the Shapiro-Wilk test for normality and for homogeneity of variance using the Bartlett test (AFDW) or Levene's test (survival) for equality of variance. Survival and dry weight met both of these assumptions. The NOAEC and LOAEC for both suggestively dose-dependent endpoints were determined using William's Multiple Comparison Test. All treatment level comparisons were made to the negative control response only. The LC₅₀ value was determined using the Probit method, while the EC₅₀ for growth was determined using nonlinear regression. Results were provided in terms of mean-measured sediment (bulk and OC-normalized, calculated based on 2.1% TOC) in the Conclusions section of the DER. Analyses were conducted using CETIS v. 1.8.7.12 with backend settings implemented by EFED on 3/25/14.

Based upon mean-measured sediment concentrations:

Survival:

LC₅₀: 275 µg ai/kg

95% C.I.: 229 to 329 µg ai/kg

Probit Slope: 2.57 (2.06 to 3.07)

NOAEC: 120 µg ai/kg

LOAEC: 220 µg ai/kg

Growth (AFDW):

EC₅₀: 92 µg ai/kg

95% C.I.: 69 to 124 µg ai/kg

Probit Slope: N/A

NOAEC: <54 µg ai/kg

LOAEC: 54 µg ai/kg

Based upon OC-normalized mean-measured sediment concentrations:

Survival:

LC₅₀: 13095 µg ai/kg TOC

95% C.I.: 10905 to 15667 µg ai/kg TOC

Probit Slope: 2.57 (2.06 to 3.07)

NOAEC: 5714 µg ai/kg TOC

LOAEC: 10476 µg ai/kg TOC

Growth (AFDW):

EC₅₀: 4381 µg ai/kg TOC

95% C.I.: 3286 to 5905 µg ai/kg TOC

Probit Slope: N/A

NOAEC: <2571 µg ai/kg TOC

LOAEC: 2571 µg ai/kg TOC

13. REVIEWER'S COMMENTS:

The reviewer's conclusions differed for the LC₅₀ from the study author's, likely due to different statistical methodology. The LC₅₀ for survival was calculated by the reviewer as 275 µg ai/kg with a C.I. of 229 to 329. This value was less than the study author's calculated value of 430 µg ai/kg. The reviewer also calculated a slightly lower EC₅₀ value (92 µg ai/kg) than the study author (110 µg ai/kg). Comparison of the treatment levels to the negative control only, rather than a pooled control, may have contributed to the reviewer's differing conclusion from the study author.

Results were provided in terms of mean-measured sediment (bulk and OC-normalized, calculated based on 2.1% TOC) in the Conclusions section of the DER.

Dosing stock solutions and treated sediment from all levels (prior to allocation into the replicate vessels) were analyzed for permethrin. Recoveries in the stock solutions ranged from 82 to 120% of nominal concentrations. Analysis of the spiked sediment following dosing and prior to allocation into the replicate exposure vessels ranged from 79 to 95% of nominal concentrations.

The analytical method used to quantify permethrin in formulated sediment was validated on April 30 to May 5, 2009. Fortified samples were extracted two to three times with methanol:purified reagent water and hexane; the extracts were combined and purified for analysis using solid phase extraction (SPE). Aliquots were analyzed using gas chromatography equipped with mass selective detection in negative chemical ionization

mode (GC-MS/NCI). The method validation established an average recovery of $93.1 \pm 8.02\%$ (CV=8.61%) for permethrin from formulated sediment fortified at 1.00 and 1000 $\mu\text{g ai/kg}$. The limit of quantitation (LOQ) was 0.475 $\mu\text{g ai/kg}$. A method validation extension was conducted in February 2010, which established an average recovery of $102 \pm 4.88\%$ (CV=4.78%) for permethrin from formulated sediment fortified at 20,000 $\mu\text{g ai/kg}$.

Overlying water was not analyzed due to the pyrethroids' strong affinity to sediment (i.e., high K_{oc} values) and regular renewal of the overlying water. Documentation supporting that only negligible amounts of pyrethroids partition to overlying water were cited (Springborn Smithers Laboratories Study Nos. 13656.6106, 13656.6107, 13656.6110, 13656.6111, and 13656.6112; Putt, 2005).

It was reported that data for permethrin concentrations in pore water and bulk sediment for pore water will be presented in a supplemental report.

In addition to total hardness and specific conductivity, total alkalinity and ammonia were determined in the overlying water of each level on Days 0 and 10. Total alkalinity ranged from 18 to 38 mg/L as CaCO_3 , and ammonia (as N) ranged from 0.54 to 2.2 mg/L on Day 0 and 1.2 to 9.9 mg/L on Day 10.

The redox potential, pH, ammonia content, dissolved organic carbon (DOC), and total organic carbon (TOC) were measured in isolated pore water at each level on Days 0 and 10. The redox potential ranged from 180 to 280 mV, the pH ranged from 6.4 to 6.8, the DOC ranged from 88 to 120 mg C/L, the TOC ranged from 100 to 160 mg C/L, and ammonia content (as N) ranged from 13 to 17 mg/L on Day 0 and 3.7 to 6.3 mg/L on Day 10.

This study was conducted in compliance with all pertinent U.S. EPA GLP regulations (40 CFR, Part 160) with the following exceptions: routine water, sediment, and food contaminant screening analyses. These analyses were performed using certified laboratories and standard validated methods.

Definitive test dates were March 19 to 29, 2010.

14. REFERENCES:

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